ORIGINAL ARTICLE 10.1111/j.1469-0691.2008.02031.x

Changes in *Streptococcus pneumoniae* serotypes causing invasive disease with non-universal vaccination coverage of the seven-valent conjugate vaccine

S. I. Aguiar, I. Serrano*, F. R. Pinto, J. Melo-Cristino and M. Ramirez on behalf of the Portuguese Surveillance Group for the Study of Respiratory Pathogens*

Instituto de Microbiologia, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

ABSTRACT

The pneumococcal seven-valent conjugate vaccine (PCV7) has been administered in Portugal since late 2001 through the private sector. To evaluate the impact of PCV7 use, the serotypes and antimicrobial susceptibility of pneumococci causing invasive disease in Portugal during 2003–2005 were determined and compared with available data for the period 1999–2002. Changes in serotype distribution compatible with the introduction of PCV7 were shown for children ≤5 years of age from 2003 onwards and for adults from 2004 onwards. PCV7 use with coverage of 43% of children with four doses in the 2004 birth cohort, although substantially below universal coverage, seems to have contributed to greatly reducing the proportion of invasive infections due to vaccine serotypes 4, 6B, 14 and 23F. Similarly, significant indirect effects on the serotype distribution of pneumococci causing infections in adults were noted, with reductions in the proportion of invasive infections caused by serotypes 4, 5 and 14. These changes were accompanied by an increase in the proportion of two non-vaccine serotypes: 19A isolates in all age groups and 7F isolates in adults. Whereas serotypes 6B, 14 and 19A were associated with multidrug resistance, isolates expressing serotypes 4 and 7F were fully susceptible for the most part. There were no changes in the proportion of resistant isolates within each serotype and, in spite of the changes in serotype prevalence, there was not an overall reduction in the proportion of infections caused by resistant pneumococci.

Keywords antimicrobial resistance, conjugate vaccine, herd immunity, invasive infections, *Streptococcus pneumoniae*

Original Submission: 10 August 2007; **Revised Submission:** 29 February 2008; **Accepted:** 5 March 2008

Edited by F. Soriano

Clin Microbiol Infect 2008; **14:** 835–843

INTRODUCTION

In early 2000, the seven-valent pneumococcal conjugate vaccine (PCV7; includes serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) was licensed in the USA, and this was followed shortly after by recommendations for the universal vaccination of infants and children under 2 years of age [1]. In the European Union, the vaccine was only licensed 1 year later, in February 2001, and actual availability in individual countries may still have required approval by the national regulatory agencies [2]. In contrast to the USA, availability was not followed shortly after by recommendations for universal vaccination. By 2003, most European countries had issued vaccination recommendations for risk groups only, although the definition of these groups varied widely among countries and could be highly targeted or include large portions of the paediatric population [3]. This situation started changing in 2005, and by January 2007, ten countries, including the UK, France and Germany, had adopted universal vaccination plans [2].

Studies in the USA, following vaccine introduction, showed a dramatic impact on the number of cases of invasive infections as well as on the distribution of the serotypes causing disease, the

Corresponding author and reprint requests: M. Ramirez, Instituto de Microbiologia, Faculdade Medicina Lisboa, Av. Prof. Egas Moniz, PT 1649-028 Lisboa, Portugal
E-mail: ramirez@fm.ul.pt

*These authors contributed equally to this work.

burden of disease caused by the vaccine serotypes being greatly reduced [1]. This decrease was observed not only in the children targeted for vaccination but also in adults, including the elderly, who have a high burden of pneumococcal disease. In fact, the largest number of cases of invasive pneumococcal disease prevented by vaccination is not among the population targeted by vaccination but among the elderly, as a consequence of herd immunity [4]. Moreover, results from other studies indicate that the vaccine also had an impact on non-invasive infections by reducing the cases of otitis media due to isolates of vaccine serotypes [5], by reducing the cases of radiologically confirmed pneumonia [6], as well as by reducing the incidence of antimicrobial-resistant pneumococcal invasive infections [7]. However, the overall decrease in the burden of pneumococcal invasive disease was accompanied by an increase in the number of cases caused by non-vaccine serotypes [5] and, in particular, the emergence of serotype 19A as an increasingly important cause of invasive infections in all age groups in the USA [8]. For unknown reasons that may be related to higher exposure, carriage rates or host susceptibility, these changes have been faster to occur and more apparent among some populations than others, as was recently demonstrated by a study of the Alaskan native population that was specifically targeted for vaccination [9].

It has been argued that pneumococcal invasive disease differs between western Europe and the USA in its incidence, which seems to be lower in Europe [10], although this finding has been questioned because of different cultural practices [11]. The prevalence of the serotypes found among invasive infections also varies between the two regions, and this affects the potential coverage of PCV7, due to the limited number of serotypes included in the vaccine. Vaccine coverage for invasive pneumococcal infections in children ≤5 years of age, estimated from the distribution of serotypes, varies from 53.8% in a Spanish study to 85% in Denmark [12], but is mostly below that estimated for the USA [13]. It remains to be shown whether the consequences of vaccination documented in the USA are due to the particular structure of the circulating pneumococcal population and whether they will be replicated in the European countries where universal vaccination was adopted.

The situation in countries where the vaccine is available but not universally administered, such as Italy [2], Spain (http://www.msc.es/ciudadanos/proteccionSalud/infancia/docs/neumo.pdf) and Portugal, is even more complex. In spite of the unquestioned efficacy of PCV7 in preventing invasive infections by vaccine serotypes among vaccinees [6], depending on the fraction of vaccinated children, one might expect a variable magnitude of reduction of the infections caused by vaccine serotypes in the overall paediatric population, and also the existence and scope of indirect effects on infections in adults. In Portugal, PCV7 has been available since June 2001, and although it is not currently included in the National Vaccination Plan, it has been administered to children through the private sector without government funding. By 2002, *c.* 33% of the children up to 3 years of age had received at least one dose of PCV7, mostly after 23 months of age [14]. More recently, a survey was conducted using a methodology that involves direct evaluation of vaccination records and that was previously shown to provide highly reliable vaccination estimates [15]. In this study, the vaccination records of approximately one-third of all children born in Portugal between 2001 and 2005 were evaluated, taking into account the currently recommended PCV7 vaccination schedule in Portugal of three doses and a booster administered at 2, 4, 6 and 18 months or at 3, 5, 7 and 18 months. The proportion of children with three doses at 12 months of age increased steadily from 23.7% in the cohort of birth in 2001 to 51.2% in the 2005 cohort, as did the proportion of children with four doses at 24 months, which increased from 20.2% in the 2001 cohort to 43.1% in the 2004 cohort (data from the 2005 cohort were not yet available) (Queirós *et al.*, 'Cobertura pela vacina pneumocócica conjugada heptavalente nas coortes de nascimento de 2001 a 2005 na região norte', available at: http://www.dgs.pt). It has previously been shown that the distribution of serotypes of pneumococci causing invasive infections were essentially stable until 2002 [16,17], establishing a baseline against which the impact of vaccination could be evaluated. The changes in the pneumococcal population causing invasive disease in Portugal from 2003 to 2005 are reported here, with particular attention given to the distribution of serotypes.

MATERIALS AND METHODS

Bacterial isolates

Since 1999, the Portuguese Surveillance Group for the Study of Respiratory Pathogens has monitored pneumococci causing invasive infections in Portugal. This is a laboratory-based surveillance system, in which 30 microbiology laboratories throughout Portugal are asked to identify all isolates responsible for invasive pneumococcal infections and to send them to a central laboratory for characterization. A case of invasive disease is defined by an isolate of *Streptococcus pneumoniae* being recovered from a normally sterile body site and does not include isolates recovered from the middle ear. Although the laboratories were contacted periodically to submit the isolates to the central laboratory, no audit was performed to ensure compliance, which may be highly variable in this type of study [18]. The isolates recovered up to 2002 were previously characterized both phenotypically and genotypically [16,17]. Only one isolate from each patient was considered and, whenever isolates from blood and cerebrospinal fluid (CSF) were available, only the CSF isolate was considered.

Serotyping and antimicrobial susceptibility testing

Serotyping was performed by the standard capsular reaction test using the chessboard system [19] and specific sera (Statens Seruminstitut, Copenhagen, Denmark). Etest strips (AB Biodisk, Solna, Sweden) were used to determine the MICs of penicillin, cefotaxime and levofloxacin, as previously described [20], and the CLSI-recommended breakpoints [21] were used to interpret MIC values. Isolates were further characterized by determining their susceptibility to erythromycin, clindamycin, vancomycin, linezolid, tetracycline, trimethoprim–sulphamethoxazole and chloramphenicol by the Kirby–Bauer disk diffusion technique, according to the CLSI recommendations and interpretative criteria [21].

Statistical analysis

Unless otherwise stated, analyses were performed using the information available for the entire period 1999–2005, taking into consideration two age groups only: children ≤5 years of age (n = 177) and adults (≥18 years, n = 1074), excluding the group of individuals aged 6–17 years (n = 47). The former group relates to current recommendations suggesting vaccination of children aged ≤5 years who attend day-care centres [22], and most children in Portugal in this age group are indeed attending day-care centres. The adult population is not vaccinated, and the group of individuals aged 6–17 years was excluded, as it presented a low incidence of infections and could include individuals vaccinated in an undefined catch-up schedule as well as non-vaccinated individuals. A global comparison of the yearly serotype distributions during the period 1999–2005 was performed through the estimation of mutual information. The value of the mutual information is a measure of dependency (similarity) between two probability distributions, expressing how much information is common to the two distributions. The absolute minimal value of mutual information is 0 and implies that the two distributions are independent. In other words, as two distributions become more similar, it becomes easier to predict one distribution by knowing the other, and this corresponds to an increase in mutual information [23]. This technique was used to identify a breakpoint delimiting two periods when the serotype distribution among invasive isolates changed most significantly. It was previously shown that the pneumococcal population causing invasive infections in Portugal was stable from 1999 to 2002 [16,17] in spite of the availability of PCV7 since June 2001. In order to evaluate the potential impact of vaccination, the breakpoint in time that maximizes the differences in serotype distribution was estimated by determining the mutual information between the distributions for every possible breakpoint year for two age groups: children ≤5 years of age and adults (≥18 years).

The vaccine impact on serotype frequency was evaluated by conditional relative risk (cRR) estimation and corresponding 95% confidence intervals computed by the Wald method [24]. The cRR was calculated relative to invasive infections caused by particular serotypes among individuals in whom invasive pneumococcal infections were detected. Individuals were considered to have been exposed to vaccine effects if the invasive infection occurred in the postbaseline period. The expression used was cRR = $(n_e/N_e)/(n_b/N_b)$, in which n_e is the number of isolates of a particular serotype or group of serotypes in the post-vaccine period, N_e is the total number of isolates in the post-vaccine period, n_b is the number of isolates of a particular serotype or group of serotypes in the baseline period, and N_b is the total number of isolates in the baseline period. Statistically significant differences in proportions of resistant isolates and associations of serotypes with particular resistant phenotypes were detected through the two-tailed Fisher exact test [24]. When all individual serotypes were simultaneously tested for association or for differences in proportion, the resulting p-values were corrected for multiple testing by keeping the false discovery rate under or equal to 0.05 through the linear procedure of Benjamini and Hochberg [25]. Other p-values indicated in the text were considered to be significant if they were lower than 0.05.

RESULTS

Isolate collection and capsular serotyping

An increase in the total number of isolates recovered in each year was noted (n = 196 in 2003, n = 256 in 2004, and n = 363 in 2005). From 2003 to 2005, 713 isolates (87.5%) were recovered from blood, 47 (5.8%) from CSF, and 55 (6.7%) from other normally sterile fluids. Forty-three isolates (5.3%) were obtained from children below 2 years of age, 47 (5.8%) from children 2–5 years of age, 32 (3.9%) from patients 6–17 years of age, and 693 (85.0%) from adults (≥18 years). All strains were confirmed to be *S. pneumoniae* by colony morphology and haemolysis on blood agar plates, optochin sensitivity, and bile solubility.

The results of serotyping of the isolates recovered from 2003 to 2005 are summarized in Fig. 1. The minima in the mutual information curves indicate the breakpoint years that reveal two time periods whose serotype distributions are more

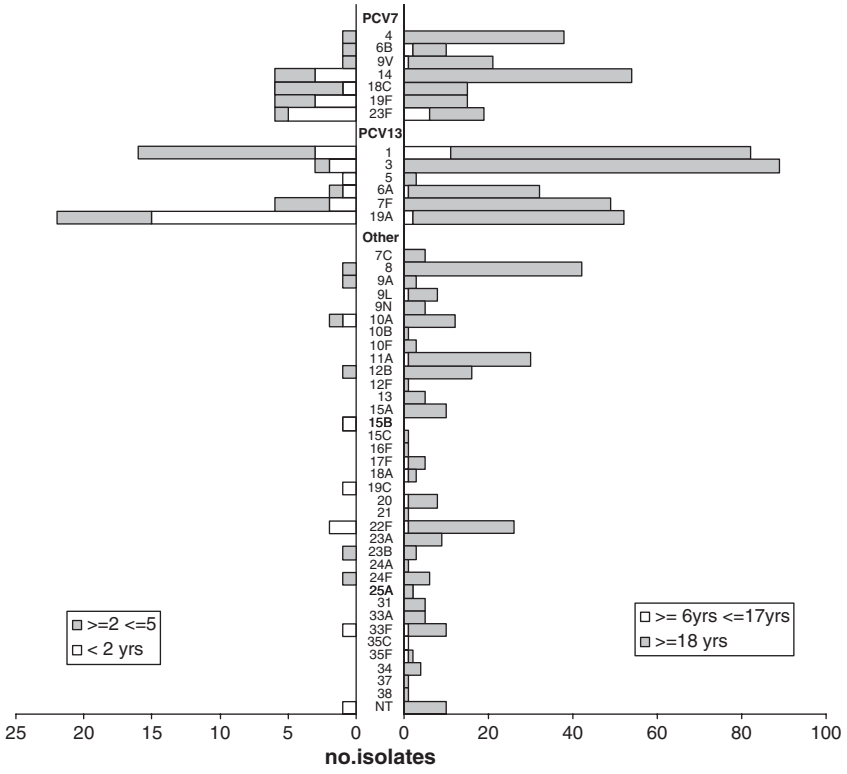

Fig. 1. Cases of invasive pneumococcal disease according to serotype and age group in Portugal (2003–2005). PCV7 indicates the serotypes included in the seven-valent conjugate vaccine. 13Pv indicates the serotypes included in the 13-valent conjugate vaccine currently under development.

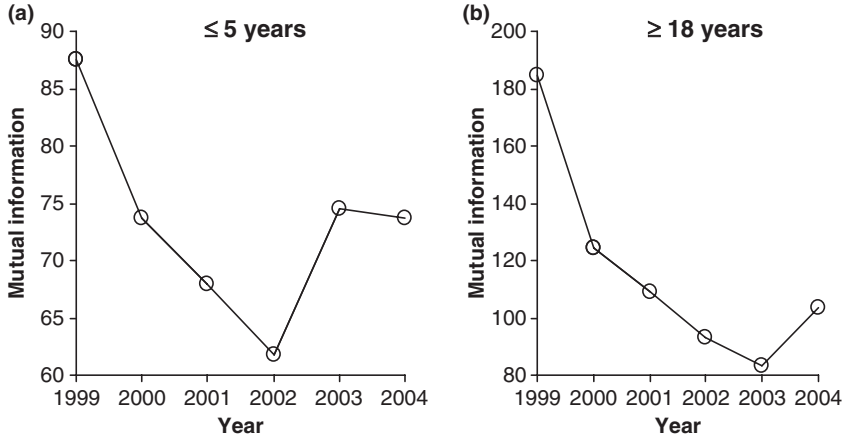

Fig. 2. Changes in serotype distribution (1999–2005). Minima in mutual information graphs indicate the years dividing the study into two periods with maximally divergent serotype distributions. (a) Analysis concerning children ≤5 years of age. (b) Analysis concerning adults (≥18 years of age).

divergent from each other (Fig. 2). The two age groups considered showed minima in different years. In the group of ≤5 year-olds, the breakpoint occurs between 2002 and 2003, but when adults are considered, the breakpoint occurs from 2003 to 2004. According to these breakpoints, from among the cases of invasive pneumococcal infections, the cRR of the infection being caused by a vaccine serotype after the breakpoint was estimated, by comparing with the same risk before the breakpoint. The conditional risk that invasive infections are caused by vaccine serotypes in children ≤5 years of age is reduced to half after 2002 (cRR = 0.50 (95% CI 0.35–0.74)). Such a reduction was expected, as PCV7 use is protecting vaccinees in this population from infections caused by vaccine serotypes. A significant reduction in the adult population after 2003 (cRR = 0.72 (95% CI 0.58–0.88)) was also noted, indicating that in this population also there were changes in serotype distribution. These two different time periods were therefore taken to define the base-

Table 1. Changes in the conditional relative risk (cRR) of invasive disease according to individual serotypes

	≤5 years of age			≥18 years of age		
Serotypes[a]	1999–2002 (*n* = 87)	2003–2005 (*n* = 90)	cRR (95% CI)	1999–2003 (*n* = 548)	2004–2005 (*n* = 526)	cRR (95% CI)
PCV7						
4	0	1	–	43	24	0.58 (0.36–0.94)
6B	8	1	0.12 (0.02–0.95)	9	5	0.58 (0.30–2.94)[b]
14	22	6	0.26 (0.11–0.62)	61	34	0.58 (0.39–0.87)
23F	15	6	0.39 (0.16–0.95)	10	11	1.15 (0.49–2.68)[b]
Others						
5	1	1	0.97 (0.06–15.21)[b]	13	1	0.08 (0.01–0.61)
19A	5	22	4.25 (1.69–10.73)	21	40	1.98 (1.18–3.32)
7F	3	6	1.93 (0.50–7.49)[b]	21	42	2.08 (1.25–3.47)

[a]Only serotypes presenting significant values in at least one of the age groups considered is indicated. Data from the 1999–2002 period were reported previously [17].
[b]cRR was not considered to be significant, as the 95% CI included 1.

line pneumococcal population and the population after an effect of vaccination is noted in the two age groups, and was used thereafter.

An analysis taking individual serotypes into account showed that not all serotypes included in PCV7 were significantly reduced after vaccine introduction. In children ≤5 years of age, serotypes 6B, 14 and 23F were reduced, whereas in the adult population, serotypes 4 and 14 showed a significant reduction in risk (Table 1). Among non-vaccine serotypes, an increased relative risk could be shown for the infections being caused by serotype 19A in both children and adults, as well as by serotype 7F, but only in adults, and a reduced relative risk of the infection being caused by serotype 5, also exclusively in adults.

Antimicrobial susceptibility

The results of antimicrobial susceptibility testing are summarized in Table 2. The serotypes most frequently found among resistant isolates are shown in Table 3. These data from the years 2003–2005 were compared with data from an earlier study from the baseline years (1999–2002 for children ≤5 years of age and 1999–2003 for those subjects 18 years old [16,17]). Possible differences in the prevalence of isolates resistant to the antimicrobial agents tested between the baseline and the period after vaccination when changes were noted in the pneumococcal population were evaluated (Fig. 2). Among children <6 years of age, a significant decrease in penicillin-non-susceptible isolates was noted, from 44.8% in 1999–2002 to 26.7% in 2003–2005 (p = 0.013). In contrast, among adults, an increase in resistance was noted between the periods 1999–2003 and 2004–2005 for several antimicrobial agents: tetracycline resistance increased from 7.7% to 12.2% (p = 0.014), erythromycin resistance from 9.7% to 13.9% (p = 0.037), and the proportion of isolates simultaneously resistant to erythromycin and non-susceptible to penicillin increased from 4.4% to 8.0%

Table 2. Antimicrobial susceptibility of isolates responsible for invasive infections (2003–2005)

	No. of resistant isolates (%)	
	≤5 years of age (*n* = 90)	≥6 years of age (*n* = 725)
PEN^{Ra}	5 (5.6)	9 (1.2)
PEN^{Ia}	19 (21.1)	108 (14.9)
CTX^{Rb}	1 (1.1)	0 (0.0)
CTX^{Ib}	3 (3.3)	8 (1.1)
LEV	0 (0.0)	1 (0.1)
ERY[c]	21 (23.3)	103 (14.2)
CLI	18 (20.0)	90 (12.4)
CHL	7 (7.8)	22 (3.0)
SXT	23 (25.6)	124 (17.1)
TET	17 (18.9)	87 (12.0)
VAN	0 (0.0)	0 (0.0)
LZD	0 (0.0)	0 (0.0)

PEN, penicillin; CTX, cefotaxime; LEV, levofloxacin; ERY, erythromycin; CLI, clindamycin; CHL, chloramphenicol; SXT, trimethoprim–sulphamethoxazole; TET, tetracycline; VAN, vancomycin; LZD, linezolid.
[a]PEN^R, penicillin-resistant; PEN^I, penicillin-intermediate. MIC range for all isolates was 0.003–12 mg/L, MIC_{50} = 0.016 mg/L and MIC_{90} = 0.5 mg/L.
[b]CTX^R, cefotaxime-resistant; CTX^I, cefotaxime-intermediate. MIC range for all isolates was 0.004–4 mg/L, MIC_{50} = 0.023 mg/L and MIC_{90} = 0.5 mg/L.
[c]The majority of macrolide-resistant isolates (87%) presented the MLS_B phenotype.

Table 3. Serotypes most frequently found among resistant isolates (all ages 2003–2005)

		No. of isolates resistant to:			
Serotype	Fully susceptible	At least one antimicrobial agent	PEN[a]	ERY	MDR
6B	2	9	6 (1)	9	9
9V	5	17	12 (2)	2	3
14	7	53	43 (4)	19	13
15A	1	9	9 (0)	9	9
19A	17	57	35 (4)	44	47
23F	4	21	18 (2)	3	5
Other[b]	524	89	18 (1)	36	30
Total	560	255	141 (14)	126	116

PEN, penicillin; ERY, erythromycin; MDR, multidrug resistance (defined as resistance to at least three antimicrobial classes).
[a]Isolates non-susceptible to penicillin are indicated. Numbers in parentheses indicate fully resistant isolates (MIC ≥2 mg/L).
[b]Other includes a total of 42 different serotypes and non-typeable isolates.

Table 4. Associations of resistance and serotype (all ages 1999–2005)

	Antimicrobial resistance phenotype[a]													
	CHL	SXT		TET		PEN		CTX	ERY		PEN + ERY		MDR	
	+	+	−	+	−	+	−	+	+	−	+	−	+	−
Serotypes			1		1		1			1		1		1
			3		3		3			3		3		3
			4		4		4			4		4		4
	6B	6B		6B		6B			6B		6B		6B	
			7F		7F		7F			7F		7F		7F
			8				8			8				
		9V				9V								
			10A				10A							
							11A			12B				
		14				14		14	14		14		14	
						15A	12B		15A		15A			
	19A	19A		19A									19A	
									19A		19A			
				19F	22F									
	23F					19A				22F				
							18C							
						23F								
				33F			22F		33A					
									33F					

CHL, chloramphenicol; SXT, trimethoprim–sulphamethoxazole; TET, tetracycline; PEN, penicillin; CTX, cefotaxime; ERY, erythromycin; MDR, multidrug resistance (defined as resistance to at least three antimicrobial classes); +, a higher than expected proportion of isolates that were non-susceptible to that particular antimicrobial among the serotypes indicated (a positive association with resistance); −, a lower than expected proportion of isolates that were non-susceptible to that particular antimicrobial agent among the serotypes indicated (a negative association with resistance).
[a]Only statistically significant associations, as measured by a two-tailed Fisher exact test with false discovery rate correction <0.05, are indicated.

($p = 0.016$). These changes could be due to alterations in the frequency of serotypes that are associated with certain antimicrobial resistance traits or to changes in the proportion of resistant isolates within a particular serotype or group of serotypes. To help distinguish between the contributions of each of these factors, the association between serotype and resistance for each of the antimicrobial classes tested was analyzed for all invasive isolates (Table 4). Serotypes 6B, 14 and 19A were significantly associated with resistance to most antimicrobial agents, in contrast to serotypes 1, 3, 4, 7F and 8, which were significantly associated with susceptibility to most antibiotic agents. Individually, none of the serotypes showed a significant change in the proportion of non-susceptible isolates between the two time periods, for any of the antimicrobial agents, either in children or in adults (two-tailed Fisher's exact test). These data indicate that the observed changes in the fraction of resistant isolates are mainly due to alterations in the relative proportions of the serotypes associated with particular resistance traits between the two periods.

DISCUSSION

Even non-universal vaccination with PCV7 has the potential to greatly change the contribution of the various serotypes to pneumococcal invasive disease. This study design does not allow an estimation of the incidence of pneumococcal invasive infections and, therefore, a quantification of the expected benefits of vaccination. A temporal trend towards more frequent blood culturing, particularly in cases of community-acquired pneumonia and bacteraemia without a focus, which are traditionally infrequent in Europe [10,18], was possibly a major factor behind the increase in the number of isolates recovered each year. The enhanced awareness of pneumococcal invasive disease, due to the availability of PCV7 and to current debates regarding the use of the 23-valent polysaccharide vaccine in Europe, may have enhanced compliance of the participating laboratories, further compounding this trend. This temporal trend would bias an estimate of the incidence of invasive pneumococcal infections; we have therefore refrained from evaluating the impact of vaccination on the burden of disease and have instead concentrated on changes in the prevalence of the different serotypes in invasive infections.

Similar to the herd effect documented in the USA [4], indirect consequences of vaccination of children were also noted in Portugal in the serotype distribution of pneumococci causing invasive infections among the adult population,

although these occurred a year later than the change in serotypes documented in children. Asymptomatic nasopharyngeal carriage of *S. pneumoniae* by young children is frequently assumed to be the main reservoir of this bacterium, and vaccination has already been shown to interfere with this process, although in Portugal this did not lead to an overall decrease in the prevalence of colonization [26]. The recurrent transmission of bacteria from children to adults is supported by the observation that the frequency of nasopharyngeal carriage is higher in adults with young children than in other adults [27], suggesting that a significant fraction of the bacteria circulating among the adult population originates in children. If that is so, it would be expected that the effect observed in the adult population would be delayed with respect to that in children, as documented here.

These changes in serotype distribution in invasive infections, which included the decrease in the proportion of vaccine serotypes 4, 6B, 14 and 23F, were accompanied by a significant increase in the proportion of two non-vaccine serotypes, 19A and 7F, of which only serotype 19A was significant in both children and adults. A post-vaccination increase in the proportion of invasive infections caused by serotype 7F among adults is so far unique to Portugal, whereas increases in all age groups of serotype 19A were also noted in several studies in the USA [8,9] and were similarly associated with multidrug resistance. For reasons that remain unclear, serotype 5 showed a reduction in cRR of infection as compared to baseline in adults in this study. Although these changes occurred at the same time that PCV7 was introduced in Portugal, and may therefore have been triggered by vaccination, the possibility that the higher yearly recovery of isolates in this survey may also have influenced serotype distribution cannot be excluded.

One of the beneficial outcomes of vaccination in the USA was the reduction of the disease burden associated with antibiotic-resistant pneumococci [7]. In agreement with these findings, the fraction of penicillin-non-susceptible pneumococci causing invasive infections in Portugal decreased in children, but this benefit was not extended to the adult population. In fact, in contrast to results from the USA [7], the fraction of tetracycline-resistant and erythromycin-resistant pneumococci causing infections in the adult population and of isolates simultaneously non-susceptible to penicillin and resistant to erythromycin increased relative to the baseline. These changes are related to an increase in the importance of serotype 19A, composed mainly of multidrug-resistant isolates (Table 3), and to the reduction of serotypes included in PCV7, namely serotypes 9V and 23F, that were found to be significantly associated with non-susceptibility to penicillin, but not simultaneously with resistance to erythromycin. In contrast, serotype 7F isolates, which were increasing in frequency exclusively in the adult population, were fully susceptible to all antimicrobial agents tested. In spite of recent reports detailing the emergence of resistance among non-vaccine serotypes, traditionally associated with susceptible isolates [28], and a general increase in resistance in non-vaccine serotypes found in other studies [29], an expansion of the proportion of resistant isolates could not be shown in any particular serotype in this study.

It has been suggested that local variations in selection pressure are the best predictor of the proportion of pneumococci resistant to β-lactams and macrolides [30]. In agreement with this suggestion, the high prevalence of penicillin-non-susceptible and erythromycin-resistant isolates observed in Portugal is paralleled by the fact that Portugal is the second largest consumer of β-lactams and the fifth largest consumer of macrolides in Europe [31]. The model of McCormick *et al.* predicts a decrease in the prevalence of penicillin-non-susceptible isolates and an overall increase in erythromycin resistance, accompanied by an increase in the prevalence of isolates that are simultaneously penicillin-non-susceptible and erythromycin-resistant [30]. This model fits the data available from several European countries participating in the European Antimicrobial Resistance Surveillance System (EARSS) project [32]. As the data analyzed included isolates up to 2002, they probably do not reflect vaccination, and the changes must be attributed to antimicrobial use. With this in mind, the strikingly increased importance of serotype 19A among both children and adults could not only have resulted from vaccine pressure but may have been accelerated or compounded by shifting trends in antimicrobial use before vaccination. Variations in the importance of the various serotypes in pneumococcal invasive infections have also been noted [33], and alterations in these

secular trends may have also influenced the magnitude of the changes described here.

The reasons behind the higher rate of isolate recovery in this study may be multifactorial, and their impact on serotype distribution is difficult to evaluate. On the whole, the data reported here suggest that the use of the conjugate seven-valent vaccine, even substantially below universal coverage such as is currently the case in some European countries, greatly reduced the proportion of invasive infections due to vaccine serotypes and resulted in significant beneficial indirect effects on the serotype distribution of pneumococci causing infections in adults. In contrast to the USA, but in agreement with previous studies of carriage in Portugal [26], a universal reduction in the proportions of infections caused by resistant pneumococci was not shown. These data highlight the importance of the local dynamics of the serotypes of pneumococci causing invasive infections in determining the momentum and characteristics of the replacement serotypes.

ACKNOWLEDGEMENTS

Members of the Portuguese Surveillance Group for the Study of Respiratory Pathogens are gratefully thanked for their valuable collaboration in this study. S. I. Aguiar, I. Serrano and F. R. Pinto were supported by grants SFRH/BD/27518/2006, SFRH/BD/14158/2003 and SFRH/BPD/21746/2005, respectively, from Fundação para a Ciência e Tecnologia, Portugal. Partial support for this work was provided by PREVIS (LSHM-CT-2003-503413 from the European Community), Fundação para a Ciência e Tecnologia (PDTC/SAU-ESA/64888/2006) and Fundação Calouste Gulbenkian.

TRANSPARENCY DECLARATION

S. I. Aguiar, I. Serrano and F. R. Pinto have no conflicts of interest to declare. M. Ramirez has consulted for Wyeth Pharmaceuticals. J. Melo-Cristino has received research grants administered through his university, and consulted for and received honoraria for speaking from Wyeth Pharmaceuticals and GlaxoSmithKline.

REFERENCES

1. Whitney CG, Farley MM, Hadler J *et al*. Decline in invasive pneumococcal disease after the introduction of protein–polysaccharide conjugate vaccine. *N Engl J Med* 2003; **348**: 1737–1746.
2. Lopalco PL, Team E. Use of 7-valent penumococcal conjugate vaccine in EU. *Euro Surveill* 2006 **11**: E061207–E061203.
3. Pebody R, Leino T, Nohynek H *et al*. Pneumococcal vaccination policy in Europe. *Euro Surveill* 2005; **10**: 174–178.
4. Centers for Disease Control and Prevention. Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease—United States, 1998–2003. *MMWR* 2005; **54**: 893–897.
5. Eskola J, Kilpi T, Palmu A *et al*. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med* 2001; **344**: 403–409.
6. Oosterhuis-Kafeja F, Beutels P, Van Damme P. Immunogenicity, efficacy, safety and effectiveness of pneumococcal conjugate vaccines (1998–2006). *Vaccine* 2007; **25**: 2194–2212.
7. Kyaw MH, Lynfield R, Schaffner W *et al*. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med* 2006; **354**: 1455–1463.
8. Pai R, Moore MR, Pilishvili T *et al*. Postvaccine genetic structure of *Streptococcus pneumoniae* serotype 19A from children in the United States. *J Infect Dis* 2005; **192**: 1988–1995.
9. Singleton RJ, Hennessy TW, Bulkow LR *et al*. Invasive pneumococcal disease caused by nonvaccine serotypes among Alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. *JAMA* 2007; **297**: 1784–1792.
10. Jefferson T, Ferroni E, Curtale F, Giorgi Rossi P, Borgia P. *Streptococcus pneumoniae* in western Europe: serotype distribution and incidence in children less than 2 years old. *Lancet Infect Dis* 2006; **6**: 405–410.
11. Salleras L, Dominguez A. *Streptococcus pneumoniae* incidence in western Europe. *Lancet Infect Dis* 2007; **7**: 242–243.
12. McIntosh ED, Fritzell B, Fletcher MA. Burden of paediatric invasive pneumococcal disease in Europe, 2005. *Epidemiol Infect* 2007; **135**: 644–656.
13. Butler JC, Breiman RF, Lipman HB, Hofmann J, Facklam RR. Serotype distribution of *Streptococcus pneumoniae* infections among preschool children in the United States, 1978–1994: implications for development of a conjugate vaccine. *J Infect Dis* 1995; **171**: 885–889.
14. de Queirós L, Castro L, Ferreira MC, Gonçalves G. Adesão às novas vacinas conjugadas—vacina anti-meningocócica e anti-pneumocócica. *Acta Med Port* 2004; **17**: 49–53.
15. Gonçalves G, Frutuoso MA, Ferreira MC, Freitas MG. A strategy to increase and assess vaccine coverage in the north of Portugal. *Euro Surveill* 2005; **10**: 98–102.
16. Serrano I, Melo-Cristino J, Carriço JA, Ramirez M. Characterization of the genetic lineages responsible for pneumococcal invasive disease in Portugal. *J Clin Microbiol* 2005; **43**: 1706–1715.
17. Serrano I, Ramirez M. The Portuguese Surveillance Group for the Study of Respiratory Pathogens, Melo-Cristino J. Invasive *Streptococcus pneumoniae* from Portugal: implications for vaccination and antimicrobial therapy. *Clin Microbiol Infect* 2004; **10**: 652–656.
18. Reinert RR, Haupts S, van der Linden M *et al*. Invasive pneumococcal disease in adults in North-Rhine Westphalia, Germany, 2001–2003. *Clin Microbiol Infect* 2005; **11**: 985–991.
19. Sorensen UB. Typing of pneumococci by using 12 pooled antisera. *J Clin Microbiol* 1993; **31**: 2097–2100.

20. Melo-Cristino J, Fernandes ML, Serrano N. The Portuguese Surveillance Group for the Study of Respiratory Pathogens. A multicenter study of the antimicrobial susceptibility of *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis* isolated from patients with community-acquired lower respiratory tract infections in 1999 in Portugal. *Microb Drug Resist* 2001; **7**: 33–38.
21. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*, 16th informational supplement. CLSI document M100-S16. Wayne, PA: CLSI, 2006.
22. Centers for Disease Control and Prevention. Preventing pneumococcal disease among infants and young children: recommendations of the Advisory Committee on Immunization Pactices (ACIP). *MMWR* 2000; **49**: 1–29.
23. Cover TM, Thomas JA. *Elements of information theory*. New York, NY: Wiley, 1991.
24. Altman DG. *Practical statistics for medical research*. Boca Raton, FL: Chapman & Hall/CRC, 1999.
25. Benjamini Y, Hochberg Y. Controlling the false discovery rate—a practical and powerful approch to multiple testing. *J R Stat Soc Ser B Stat Methodol* 1995; **57**: 289–300.
26. Frazão N, Brito-Avô A, Simas C *et al.* Effect of the seven-valent conjugate pneumococcal vaccine on carriage and drug resistance of *Streptococcus pneumoniae* in healthy children attending day-care centers in Lisbon. *Pediatr Infect Dis J* 2005; **24**: 243–252.
27. Hendley JO, Sande MA, Stewart PM, Gwaltney JM Jr. Spread of *Streptococcus pneumoniae* in families. I. Carriage rates and distribution of types. *J Infect Dis* 1975; **132**: 55–61.
28. Porat N, Arguedas A, Spratt BG *et al.* Emergence of penicillin-nonsusceptible *Streptococcus pneumoniae* clones expressing serotypes not present in the antipneumococcal conjugate vaccine. *J Infect Dis* 2004; **190**: 2154–2161.
29. Farrell DJ, Klugman KP, Pichichero M. Increased antimicrobial resistance among nonvaccine serotypes of *Streptococcus pneumoniae* in the pediatric population after the introduction of 7-valent pneumococcal vaccine in the United States. *Pediatr Infect Dis J* 2007; **26**: 123–128.
30. McCormick AW, Whitney CG, Farley MM *et al.* Geographic diversity and temporal trends of antimicrobial resistance in *Streptococcus pneumoniae* in the United States. *Nat Med* 2003; **9**: 424–430.
31. Goossens H, Ferech M, Van der Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* 2005; **365**: 579–587.
32. Bruinsma N, Kristinsson KG, Bronzwaer S *et al.* Trends of penicillin and erythromycin resistance among invasive *Streptococcus pneumoniae* in Europe. *J Antimicrob Chemother* 2004; **54**: 1045–1050.
33. Finland M, Barnes MW. Changes in occurrence of capsular serotypes of *Streptococcus pneumoniae* at Boston City Hospital during selected years between 1935 and 1974. *J Clin Microbiol* 1977; **5**: 154–166.